Culturing Chick Embryonic Cardiac Myocytes and Fibroblasts

Materials

1. Culture medium: F12K without glutamine, 3% FCS, 100 units/ml, 100 ug/ml streptomycin (myocyte medium, also for mixed culture); and F12K with glutamine, 5-10% FCS, 100 units/ml penicillin, 100 ug/ml streptomycin (fibroblast medium), on ice. FCS should be screened for its ability to support plating and myocyte beating.

2. Dissection tools: 1 pair of large scissors; 2 pairs fine scissors; 2 pairs of coarse forceps; 2 pairs of fine forceps; soaking in 95% EtOH.

3. Saline G - Ca^{2+} and Mg^{2+} free.

4. Enzyme - 0.125% trypsin + 0.05% collagenase in saline D. These concentrations work well for 6-7 day embryos. Older embryos need 0.25% trypsin and a higher concentration of collagenase. With 6-day and younger embryos, no collagenase is necessary. Thaw the solution and put in the incubator at 34 or 39^{o}C.. Need 1 ml/heart (for myocytes) or 0.5 ml/heart (for fibroblasts).

5. F12K with glutamine + 20% FCS + 1% penicillin/streptomycin. Same volume as trypsin-collagenase (~5 ml), in a 15 ml conical tube and keep on ice.

6. Chamber dishes. Place 1.5 ml appropriate medium on each dish and incubate at 34^{o}C.

7. Preplate for myocyte culture. Add 1 ml of fibroblast medium to a 60 mm dish and put in the 34 or 39^{o}C incubator.

8. 2x 100 mm sterile petri dishes - glass or plastic - on ice.

9. Chick embryos, 7-day is optimal, but 6- or 8-day embryos are also useable. Need 1-2 embryos for each chamber dish: one per dish if no preplating, 2 per dish with preplating. Each heart yields about 105 cells (myocytes plus fibroblasts).

10. 15 ml conical centrifuge tubes.

Procedure (perform in a still air hood)

1. Spray eggs 2x with 70% EtOH.

2. Wipe top surface with 95% EtOH thoroughly. Let dry completely.

3. Put 1-2 ml Saline G in one of the petri dishes to pool hearts and keep on ice until use.

4. Remove embryos to the sterile 100 mm petri dish without saline G. It is easier to grab the embryos at feet. Dissect each embryo on ice immediately if you are slow. If you are fast, you can remove all the embryos first and dissect them later.

5. Behead embryos. Dissect and remove the whole heart using a clean pair of fine scissors and forceps and place in the dish with saline G. Hearts can be identified based on the presence of blood, beating, and associated blood vessels.

6. Cut each heart through the ventricle to drain any remaining blood.

7. Pipet off saline G. Reapply 1-2 ml saline G, rinse, and pipet off.

8. Repeat rinse one more time, or until saline G looks fairly "clean" (free of blood). More rinses necessary for myocytes. Leave some saline G after the final rinse, about 0.2 ml per heart.

9. Using a clean pair of fine scissors, chop hearts to small but uniform size - about that of sand particles. Be careful not to chop too long, because cells can be damaged.

10. Transfer cells to a 15 ml conical centrifuge tube. Use a small amount of saline to rinse the dish. Repeat the rinse if necessary. The total volume should be close to 0.5 ml per heart.

11. Trypsinization #1: mix the enzyme solution with a Pasteur pipet. Add 0.5 to 1 ml enzyme to the tube. Mix by shaking. Make sure tissues are not sticking to the side of the tube. Place the tube in the 34 or 39^{o} incubator for a total of ~5min. Shake or vortex the tube every 2-3 min. Do not let solution sit too long and become too turbid (will lose a lot of cells). Keep the remaining enzyme solution warm.

12. Remove the tube from the incubator and vortex at 1/2 speed. Length of time vortexing depends on the length of trypsinization (short vortexing for long trypsinization). Typically 15-20 sec. Shake pieces off the side and let settle for 1-2 min.

13. Pipet off supernatant and discard.

14. Trypsinization #2: add 0.5 to 1 ml enzyme to the settled pieces. Vortex at 1/2 speed for 10-15 sec. Incubate for ~5min as in step 11.

15. Remove the tube from the incubator and vortex at full speed for 10-15 sec. Shake pieces off the side and let settle for 1-2 min. Suspension never looks as cloudy as after the first trypsinization because of the much small number of red blood cells.

16. Transfer the supernatant to the tube with cold medium containing 20% FCS. Avoid clumps.

17. Repeat the trypsinization on the settled materials. The enzyme solution should be divided among the number of treatment. For myocytes, repeat 3-5 times. For fibroblasts, repeat 1-2 times. Accumulate all supernatant in the same tube with 20% FCS.

18. Vortex hard at the end of last trypsinization for 15 sec to try and break up clumps. Pipet up and down to break up clumps even more.

19. Centrifuge in table-top centrifuge on high setting (not quite max speed) for at least 5 min.

20. Remove supernatant and discard. Be sure not to disturb pellet.

21. Resuspend pellet in ~1 ml cold medium (for 4 hearts). Break up pellet completely by pipeting.

22. For myocytes only, transfer the suspension to the preplate with warm fibroblast medium. Incubate for 45-50 min. Collect the supernatant in a 15 ml conical tube.

23. Transfer all suspension to plates/dishes. Usually innoculum size is ~0.2 ml per chamber dish or 0.3 ml per 60 mm dish. For fibroblasts (without preplating), each chamber dish should contain ~6 x 104 cells. For myocytes, count cells in the supernatant after preplating and plate at 4 x 104 cells per chamber dish.

24. Change medium 2-3 hr later for fibroblasts; 24 hr later for myoblasts. Use appropriate media. Feed cells every 2-3 days.

25. Cardiac myocytes need 3-5 days to spread out.
